FORMULATION AND CHARACTERIZATION OF A NANOEMULSION-BASED MOUTHWASH INCORPORATING *ALPINIA OFFICINARUM* EXTRACT WITH *SANGU PARPAM*FOR GENERAL DENTAL HYGIENE

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ABSTRACT

Oral diseases, fueled by harmful bacteria, pose a significant global health challenge, impacting human well-being. Dentistry frequently recommends mouthwashes to prevent and treat various oral conditions. The therapeutic advantages of active plant ingredients are well-established, making them popular for treating ailments at home and in clinics. Herbs are valuable resources in traditional medicines and are utilized in diverse nano formulations. This study aims to create and characterize a cost-effective Nano emulsion-based mouthwash incorporating *Alpinia officinarum* with *Sangu parpam* to maintain general dental hygiene. Various methods, including probe sonication and ultrasonication, were used to prepare nano emulsions, with formulation methods evaluated for efficiency. Compatibility was assessed using FTIR analysis, and UV spectroscopy was employed to determine drug content. pH and viscosity were measured using standard techniques. Droplet size distribution and zeta potential were analysed, and surface morphology was examined via SEM. *In-vitro* growth inhibition studies were conducted using agar disc diffusion and checkerboard assay methods. Statistical analysis, including ANOVA, was performed to assess differences between formulations. Stability studies under different conditions confirmed the safety and efficacy of the formulations over time. The optimized formulations exhibited spherical shape, with *Alpinia officinarum* and *Sanguparpam* content meeting desired ranges. pH remained neutral, and viscosity was consistent across formulations. Antibacterial activity was observed in all formulations, with selected formulations demonstrating superior results. Stability tests indicated long-term stability under refrigerated and room temperature conditions. The research highlights the potent antibacterial efficacy of the nano emulsion mouthwash, offering a promising option for oral care with precise targeting and safety features, potentially leading to innovative antibacterial agents.

Keywords: Nanoemulsion Mouthwash Alpinia officinarum Sangu Parpam Antibacterial activity

1. INTRODUCTION

Understanding the causes of tooth decay and gum diseases empowers individuals to safeguard their oral health effectively. Tooth decay, fueled by bacterial action on sugars, highlights the importance of mindful dietary choices. [1] Bacterial biofilm formation on teeth exacerbates these conditions, emphasizing the need for proactive oral hygiene Periodontal diseases, stemming from bacterial infections around teeth, pose significant risks, with gingivitis potentially progressing to periodontitis if untreated. Diagnosis involves thorough examination, often supplemented with imaging for comprehensive assessment. [2,3] Treatment strategies, tailored to individual needs, may include antibiotics, antifungals, or antivirals alongside diligent oral hygiene practices. Mouthwashes, with ingredients like chlorhexidine, complement oral care routines, aiding in bacterial control and plaque reduction. Collaboration with dental professionals ensures personalized care, promoting long-term oral health and overall well-being. By embracing preventive measures and adhering to treatment plans, individuals can effectively protect their smiles and maintain vibrant oral health. [4,5] Natural herbal mouthwashes offer a compelling alternative to synthetic or cosmetic mouthwashes due to their reliance on time-tested ingredients, gentle formulation suitable for sensitive mouths, and absence of harsh additives. These herbal solutions harness the inherent antibacterial properties of natural ingredients without causing dryness, meeting the rising demand for effective yet gentle oral care products. As the market increasingly recognizes the drawbacks of synthetic options, the appeal of herbal mouthwashes continues to grow. Amidst the two main categories of mouthwashes available—cosmetic and therapeutic—it's evident that therapeutic mouthwashes containing synthetic compounds like chlorhexidine (CHX) offer substantive antimicrobial benefits but come with potential side effects such as staining and altered taste sensations. [6,7] In contrast, herbal mouthwashes utilize natural herbs like mint, tea tree oil, and eucalyptus, renowned for their cleansing and healing properties, offering a holistic approach to oral hygiene without the drawbacks associated with synthetic alternatives. In addressing the pressing need for oral health treatments, especially against pathogens like *Staphylococcus aureus*, herbal mouthwashes present a

promising avenue. [8-10] Traditional plants like *Alpinia officinarum* and *Sangu Parpam* are rich sources of bioactive compounds with proven antimicrobial and anti-inflammatory properties, making them ideal candidates for inclusion in mouthwash formulations. These natural ingredients not only combat oral bacteria but also contribute to overall oral health by preventing issues like bad breath, gingivitis, and plaque formation. By tapping into the therapeutic potential of natural ingredients, herbal mouthwashes offer a safe and effective solution for maintaining oral hygiene, catering to the growing preference for natural products among consumers. However, despite their promising benefits, the utilization of these natural sources in mouthwash formulations remains underexplored, highlighting the need for further research to unlock their full potential in promoting oral health and well-being. [11-15] Nano emulsion systems have emerged as a revolutionary approach for targeted drug delivery in pharmaceutical applications. These systems, characterized by their nano-sized emulsions, are engineered to enhance the delivery efficiency of active pharmaceutical ingredients (APIs). By seamlessly combining two immiscible liquids into a single phase with the aid of emulsifying agents, nano emulsions ensure the uniform dispersion of APIs, facilitating their effective delivery to targeted sites within the body. Nano emulsions offer distinct advantages such as small droplet size, high kinetic stability, large surface area, and low viscosity. These properties make them ideal carriers for pharmaceuticals, enabling precise control over drug release and enhancing bioavailability. Additionally, nano emulsions can be tailored for various administration routes, including topical, oral, and parenteral, further expanding their applicability in drug delivery. In the context of oral care, nano emulsion-based formulations present a promising avenue for mouthwash development. Nano emulsions offer superior stability and clarity compared to traditional emulsions, making them ideal for oral applications. By incorporating natural ingredients such as *Alpinia officinarum* extract and *Sanguparpam*, nano emulsion mouthwashes can provide effective antibacterial action while improving overall dental hygiene. The formulation and characterization of a nano emulsion-based mouthwash incorporating these botanical extracts hold great potential for treating oral antibacterial disorders and enhancing dental hygiene. These mouthwashes offer advantages such as enhanced penetration, stability, and solubility of active ingredients, aligning with the growing demand for herbal remedies in dental care. [16-18]

2. MATERIALS

Clove oil, Tween 80, and PEG 400 were sourced from Iso Chem Laboratories and Global Chemicals. Glycerine was obtained from Spectrum Reagents & Chemicals Pvt. Ltd.; menthol and ethanol were acquired from Krishna Pharma. Sodium saccharin and sodium benzoate were supplied by Qualigens Fine Chemicals. Botanical extracts including Alpinia Officinarum and Sangu Parpam were sourced from SKM Siddha and Ayurveda, respectively. Amaranth and honey were procured from Global Chemicals and Dabur India Ltd., respectively. Equipment and apparatus utilized included a Soxhlet apparatus, glass tubes, petri plates, and glass rods from Borosil, ultrasonicators from Labman and Sonics Materials, a magnetic stirrer from Remi, and a water bath from Sigma. Other equipment comprised a pH meter (Elico L1120), viscometer (Brookfield), Malvern Zetasizer (Microlabs), and scanning electron microscopy equipment from Thermo Fisher Scientific. Laboratory instruments included laminar airflow, an incubator, and a sterile disc provided by Labline, Scientek, and Himedia, respectively. The experimental setup also included a DBK zone reader from DBK Instrument.

3. METHODS

The botanical extracts, Alpinia officinarum and Sangu parpam, underwent thorough organoleptic evaluation and solubility assessments. Alpinia officinarum was extracted using a Soxhlet apparatus with ethanol, followed by purification and drying. Fourier-Transform Infrared Spectroscopy (FTIR) was employed to analyse drug compatibility. In vitro growth inhibition studies were conducted for both extracts via qualitative and quantitative antibacterial assays to determine optimal concentrations for nano emulsion preparation. Nano emulsions were prepared using high-energy and low-energy methods and characterized for physical attributes, pH, and viscosity. Mouthwash formulations were developed using nano emulsions with varying concentrations of key ingredients, ensuring optimal taste, efficacy, and stability. Comprehensive analysis and validation supported the formulation process.

3.1 PREPARATION OF HERBAL MOUTHWASH FORMULATION [19]

Tab: 1 Preparation of Mouthwash for Nano emulsion using probe sonication

Mouthwash formulation	PEG400(g)	Glycerine(ml)	Ethanol(ml)	Water(ml)
F1B1	3	3	1	3
F1B2	4	4.5	0.5	1
F1B3	4.5	5	0.5	0
F1B4	3	3	0	4

Four different solvent mixtures (B1-B4), each totalling 10 ml, were prepared as mouthwash base formulations using polyethylene glycol 400, glycerine, ethanol, and distilled water. A 40 ml nano emulsion (F1) was prepared through probe sonication and then divided into four equal parts of 10 ml each. Each part was combined with one of the four different bases (B1, B2, B3, and B4). All formulations included the same concentration of other excipients, such as colouring agents, flavouring agents, preservatives, and sweetening agents.

Tab: 2 Preparation of Mouth wash for Nano emulsion using Magnetic stirring

Mouthwash formulation	PEG400(g)	Glycerin(ml)	Ethanol(ml)	Water(ml)
F2B1	3	3	1	3
F2B2	4	4.5	0.5	1
F2B3	4.5	5	0.5	0
F2B4	3	3	0	4

Four different 10 ml solvent mixtures (B1-B4) were prepared as mouthwash bases using polyethylene glycol 400, glycerine, ethanol, and distilled water. A 40 ml nano emulsion (F2) was prepared via magnetic stirring and divided into four 10 ml portions, each combined with one of the bases (B1, B2, B3, and B4). All formulations included the same concentration of other excipients, such as colouring agents, flavouring agents, preservatives, and sweetening agents.

Tab: 3 PreparationofMouthwashforNanoemulsionusingUltrasonicationandMagnetic stirring

Mouthwash formulation	PEG400(g)	Glycerin(ml)	Ethanol(ml)	Water(ml)
F3B1	3	3	1	3
F3B2	4	4.5	0.5	1
F3B3	4.5	5	0.5	0
F3B4	3	3	0	4

Four different 10 ml solvent mixtures (B1-B4) were prepared as mouthwash bases using polyethylene glycol 400, glycerine, ethanol, and distilled water as shown in Table 3. A 40 ml nano emulsion (F3) was prepared using ultrasonication and magnetic stirring, then divided into four 10 ml portions. Each portion was combined with one of the bases (B1, B2, B3, and B4). The formulation maintained uniform concentrations of sodium benzoate (preservative), sodium saccharin (sweetening agent), menthol (flavouring agent), and amaranth (colouring agent) across all 12 samples.

3.2 Evaluation of Mouthwash

1. **Physical Examination** [20]

The colour and odour of the mouthwash formulations were assessed through visual examination.

2. **Compatibility Study** [21]
Compatibility studies were conducted on three selected mouthwash samples using Fourier-transform infrared (FTIR) spectroscopy with the KBr disc method. Samples were scanned from 4000 to 400 cm^-1 to identify functional groups and detect any interactions among formulation materials.[22]

3. **Drug Content**
The percentage drug entrapment efficiency of the nano emulsion formulation was determined using a UV-visible spectroscopic method. Each 1 ml sample was centrifuged at 3500 rpm for 30 minutes. The supernatant was then diluted with 10 ml of distilled water, and the drug concentration was measured at 270.50 nm. Results were averaged from triplicate measurements.[23]

4. **pH Measurement**
The pH of the mouthwash formulations was measured using a digital pH meter calibrated with standard buffer solutions at pH 4, 7, and 9.2. Approximately 10 ml of each nano emulsion-loaded mouthwash formulation (F1 to F3 with bases B1 to B4) was analyzed. This provided a comprehensive understanding of how base concentration variations affect the pH of the mouthwash, crucial for optimizing formulation stability, compatibility with oral tissues, and antimicrobial efficacy.[24]

5. **Viscosity**
The viscosity of the formulated mouthwash was measured using a Brookfield viscometer at 100 rpm with spindle No. 62.

6. **Droplet Size Distribution, Zeta Potential, and Stability** [25]
These parameters were measured using a Malvern Zetasizer. The selected formulation was diluted 1:10 with water and injected into the sample cell. Measurements were performed in triplicate to calculate the zeta potential based on electrophoretic mobility.[26]

7. **Surface Morphology**
The selected liquid samples were lyophilized and analyzed using scanning electron microscopy (SEM) to determine external morphology, chemical composition, and crystalline structure. SEM images were obtained with magnifications ranging from 20X to 200kX.[28]

8. **Microbial Growth Test**
Mouthwash formulations containing Alpinia officinarum and Sanguparpam nano emulsions were tested for microbial growth using the disc diffusion method on Mueller Hinton Agar. Plates were incubated at 37°C for 24 hours, and microbial growth was compared to a standard.[27]

9. **Stability Test**
Stability studies were conducted under refrigerated (2-8°C) and room temperature (37°C) conditions for one month. Parameters such as physical appearance, pH, and viscosity were evaluated to detect any signs of instability or degradation. Changes in these attributes could indicate instability or degradation, affecting product quality.[29]

10. **Statistical Analysis**
Statistical analysis was performed using GraphPad Prism version 10.0 software to compare the zone of inhibition (ZOI) values of optimized mouthwash formulations with those of the standard and solvent. A parametric one-way ANOVA test was conducted to determine significant differences among groups, with significance set at $p < 0.05$.

4. RESULTS AND DISCUSSION

a. Organoleptic properties:

Tab: 4 Physical descriptions of Drugs

Properties	**Description of *ALPINIAOFFICINARUM***	**Description of *SANGUPARPAM***
Color	Reddish-whitecolor.	Light yellow
Aroma	Slightly sweet aroma	Scent
Solubility	Soluble in ethanol	Soluble in Honey.

Taste	Peppery, Pungent, and slightly bitter	Unique and Delightful
Size	1to 2 meters	0.5 to 2 Microns
Consistency	Fine Powder	Fine powder

b. COMPATIBILITY STUDIES:

COMPATIBILITY STUDIES UTILIZING FTIR FOR *ALPINIA OFFICINARUM*

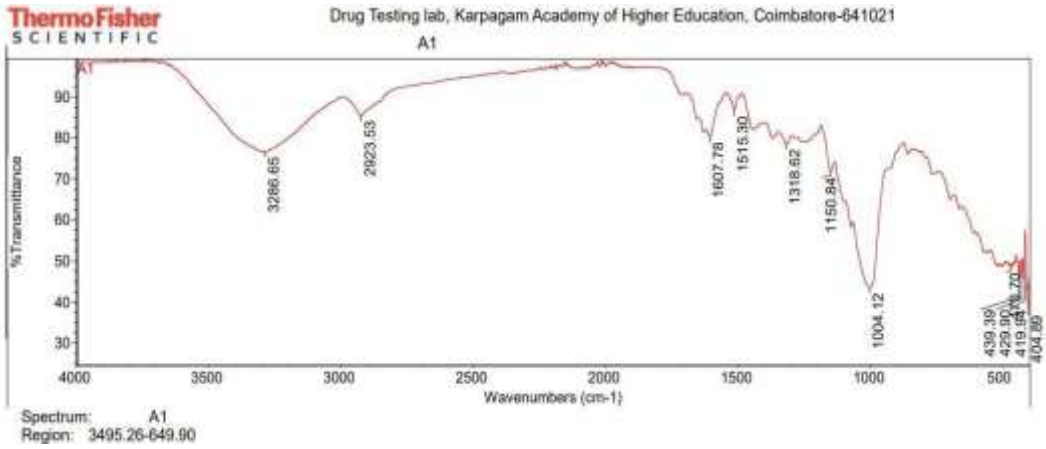


Fig:1 FT-IR for *ALPINIA OFFICINARUM*

Tab: 5 Interpretation of FT-IR for *ALPINIA OFFICINARUM*

S.no	Wavenumber (cm^{-1})	Wave range (cm^{-1})	Functional group
1	3286.65	3000-3500	N-H Stretching
2	2923.53	3000-2850	CH Stretching
3	1607.78	1650-1550	C=Stretching

COMPATIBILITY STUDIES UTILIZING FTIR FOR *SANGU PARPAM*

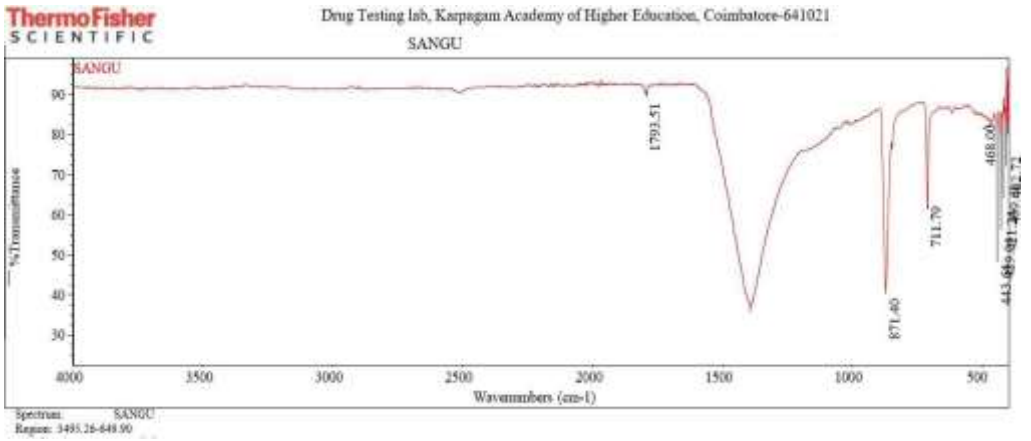


Fig:2 FT-IR for *SANGU PARPAM*

Tab: 6 Interpretation of FT-IR for *SANGU PARPAM*

S.NO	Wavenumber (cm^{-1})	Waverange (cm^{-1})	Assignment
1	1793.51	1797.90-1790	C-Nstreching
2	871.40	1100-800	C-H bending
3	711.79	710-510	O-Hstreching

DRUG– EXCIPIENT MIXTURE

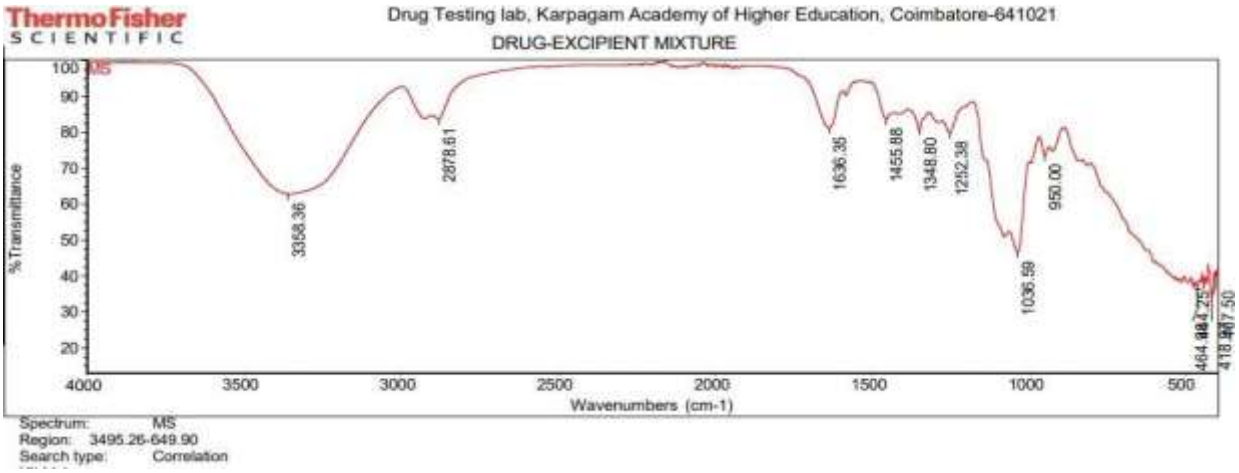


Fig:3 FT-IR for Drug excipient mixture

Tab: 7 Interpretation of FT-IR for Drug excipient mixture

S.NO	Wave number (cm^{-1})	Wave range (cm^{-1})	Assignment
1	3358.36	3358.53	OH stretching
2	2878.61	3000- 2850	CH stretching
3	1636.35	1650- 1550	C=C stretching

c. EXTRACTION OF *ALPINIA OFFICINARUM*

Ethanol proves highly effective as a solvent for extracting Alpinia officinarum, while the Soxhlet apparatus, a method extensively detailed in literature, is routinely employed for this purpose due to its efficiency in recovering active compounds from plant materials. The determination of percentage yield from the extraction process yields pivotal concentration data for the ethanol extract of Alpinia officinarum. This concentration data is essential for subsequent dilution steps, particularly in the context of antimicrobial assays, where precise control over concentration is crucial for adjusting the extract to desired levels. This meticulous control ensures consistent and reliable results in antimicrobial activity testing. Moreover, such precision enhances the accuracy and reproducibility of parameters such as the zone of inhibition, thereby bolstering the reliability of antimicrobial assays.

d. *IN-VITRO* GROWTH INHIBITION STUDY

Zone of inhibition of the drug by Qualitative antibacterial assay

Tab:8 Zone of inhibition for the drug *Alpinia officinarum*

S.NO	**$D_1$20µl**	**$S_1$20µl**
1	41.1 mm	33.4 mm
2	44.1 mm	33.6 mm
3	45.5 mm	33.6 mm
	130.7/3=44 mm	100.6/3=34 mm
	$D_1$20µl - $S_1$20µl 44 – 34 = 10 mm	

Tab:9 Zone of inhibition for the drug *Sangu parpam*

S.NO	**$D_2$20µl**	**$S_2$20µl**
1	47.6 mm	40.4 mm
2	46.4 mm	40.5 mm
3	46.4 mm	40.4 mm
	140.4/3=47 mm	121.3/3=40 mm
	$D_2$20µl - $S_2$20µl 47-40 = 7mm	

Tab: 10 Zone of inhibition for the combination of drug

S.NO	**D1+D220µl**	**S1+S220µl**
1	46.6 mm	36.7 mm
2	50.2 mm	38.2 mm
3	49.5 mm	38.2 mm
	147/3 = 49 mm	113.1/3 = 38 mm
	D_1+D22µl S_1+$S_2$20µl = 11mm	

Tab:11 Determination of appropriate dosage of the drug for preparation of nano emulsion by quantitative assay

S.no	**D1 (1mg/ml)**	**D1 (1.3mg/ml)**	**D1 (2mg/ml)**	**D2 (1mg/ml)**	**D2 (1.3mg/ml)**	**D2 (2mg/ml)**	**S1**	**S2**	**Std**
1	29 mm	30 mm	30 mm	23 mm	24 mm	29 mm	21mm	17mm	20mm
2	29 mm	31mm	30 mm	22 mm	25 mm	29 mm	21mm	17mm	20mm
3	27 mm	30 mm	31 mm	23 mm	25 mm	31 mm	21mm	17mm	20mm
	=28.3mm	=30.3mm	=30.3mm	=23mm	=25mm	=30mm	=21mm	=17mm	20mm

Where,
D1=*Alpinia officinarum*, D2 = *Sanguparpam*, S1=Ethanol, S2 = Honey

COMPARISON OF DRUG AND SOLVENT ZONE OF INHIBITION FOR DOSAGE DETERMINATION

Fig:4 Zone of inhibition in response to conc. of Drug 1

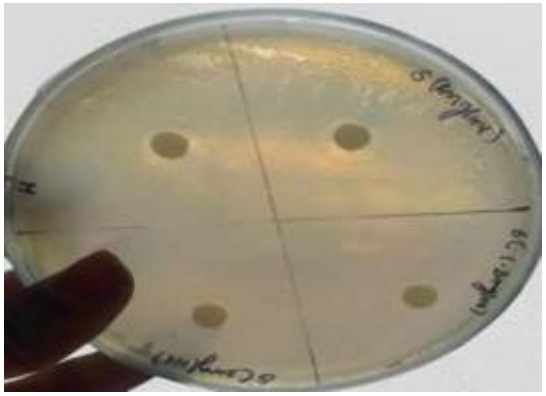


Fig:5 Zone of inhibition in response diff to diff conc. of Drug 2

e. PREPARATION OF NANOEMULSION

Various techniques were employed for the preparation of nano emulsions, including ultrasonication, magnetic stirring, probe sonication, and combinations thereof. Subsequently, nano emulsions underwent comprehensive consistency assessments following initial preparation. Evaluation of nano emulsions prepared using all three methods focused on their physical attributes, with consistency and stability being the key areas of interest. Meticulous examination revealed that nano emulsions produced by all methods demonstrated good consistency and stability. This uniformity in formulation across different methods suggests their adaptability for accommodating various base concentrations in mouthwash formulations, indicating promising prospects for practical applications.

f. CHARACTERIZATION OF NANOEMULSIONS

Tab:12 CHARACTERIZATION OF NANOEMULSIONS

S.no	Formulation	Color	Aroma	pH	Viscosity
1	Probe sonication	Sunset Yellow	Delightful fragrance.	7.10	3.16 cp
2	Magnetic stirring	Sunset Yellow	Delightful fragrance.	6.74	3.23 cp
3	Ultrasonication and Magnetic stirring	Sunset Yellow	Delightful fragrance.	7.20	3.46 cp

g. EVALUATION OF MOUTHWASH

The final mouthwash formulation exhibited a delicate pale pink hue due to the addition of the coloring agent amaranth, adhering to regulatory guidelines, ensuring both aesthetic appeal and safety compliance. Additionally, the mouthwash, enriched with nano emulsion loading, emitted an enchanting fragrance, potentially masking any unpleasant tastes or odors associated with its active ingredients, thereby enhancing its acceptability and usability.

Tab:13 Evaluation of mouth wash

S.no	D1 –S1 (1mg/ml)	D1–S1 (1.3mg/ml)	D1–S1 (2mg/ml)	D2–S2 (1mg/ml)	D2–S2 (1.3mg/ml)	D2–S2 (2mg/ml)	S1	S2	Std
1	29-21 = 8mm	30-21 = 9mm	30-21 = 9mm	23-17 = 6mm	24-17 = 7mm	29-17 = 12mm	21	17	20
2	29-21 = 8mm	31-21 = 10mm	30-21 = 9mm	22-17 = 5mm	25-17 = 8mm	29-17 = 12mm	21	17	20
3	27-21 = 6mm	30-21 = 9mm	31-21 = 10mm	23-17 = 6mm	25-17 = 8mm	31-17 = 14mm	21	17	20

COMPATABILITY STUDIES(FTIR) FOR FORMULATIONS

FT-IR data of F2B2

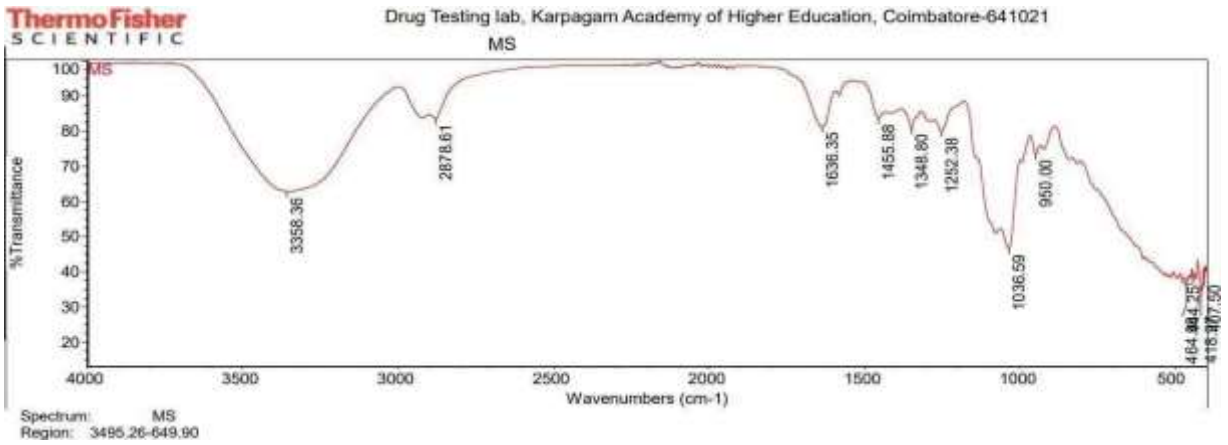


Fig:6 FT-IR for F2B2

Tab:14 Interpretation of FT-IR for F2B2

S.no	Wavenumber (cm^{-1})	Wave range (cm^{-1})	Assignment
1	3358.36	3358.53	OH stretching
2	2878.61	3000-2850	CH stretching
3	1636.35	1650-1550	C=H Stretching

FT-IR data of F3B4

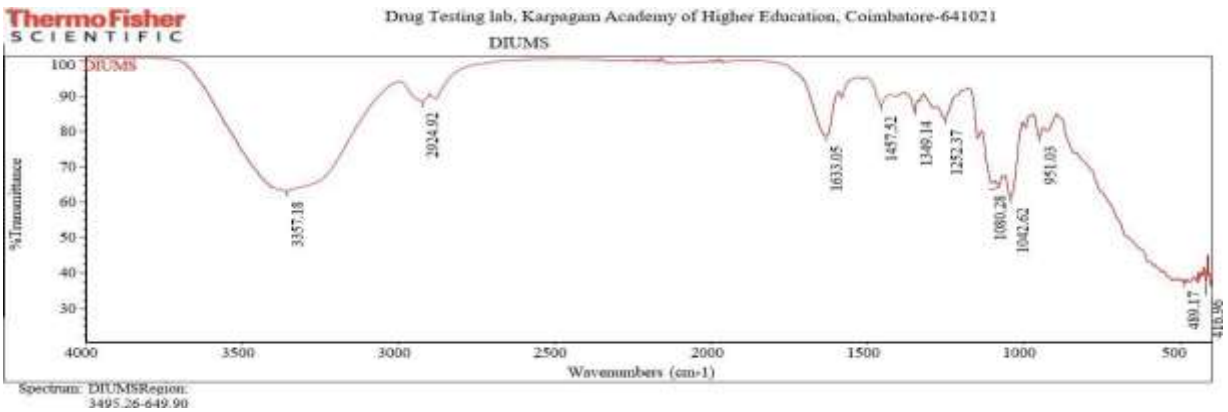


Fig:7 FT-IR for F3B4

Tab:15 Interpretation of FT-IR for F3B4

S.no	Wavenumber (cm^{-1})	Wave range (cm^{-1})	Assignment
1	3357.18	3550-3200	OH stretching
2	2924.92	3000-2850	CH stretching
3	1633.05	1650-1550	C=C Stretching

FT-IR data of F1B4

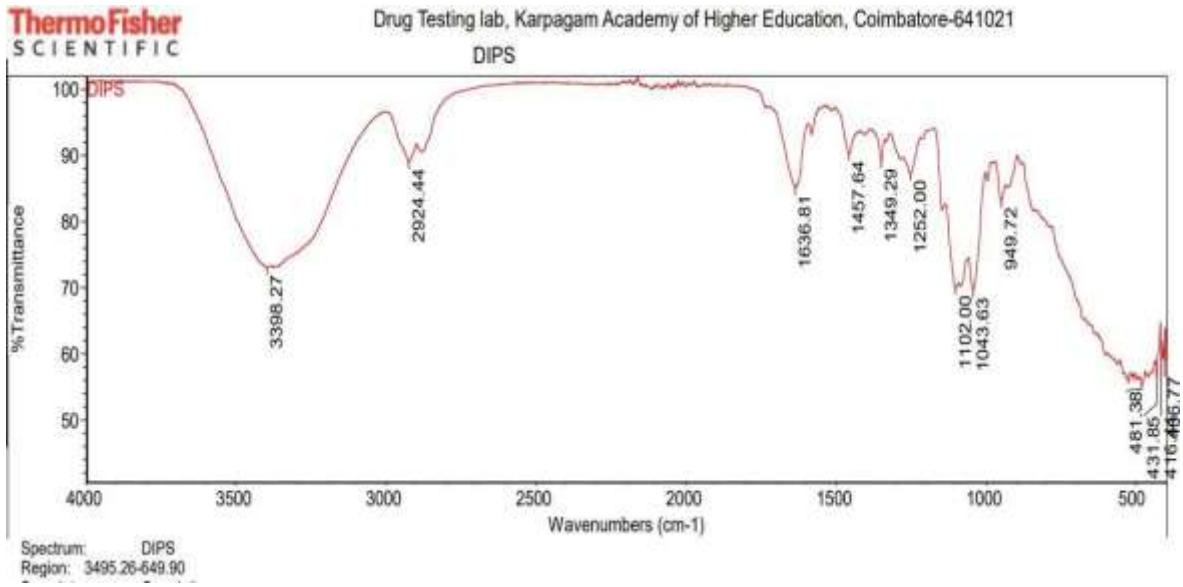


Fig:8 FT-IR for F1B4

Tab:16 Interpretation of FT-IR for F1B4

S.no	Wavenumber	Wave range (cm^{-1})	Assignment
1	3398.27	3500-3000	N-Stretching
2	2924.44	3000-2850	CH stretching
3	1636.81	1650-1550	C=C stretching

h. DRUG CONTENT

Tab:17 CALIBRATION CURVE OF *ALPINIA OFFICINARUM*

S.NO	CONCENTRATION (µg/ml)	BSORBANCE At 269.50 nm
1	0	0
2	2	0.143
3	4	0.268
4	6	0.421
5	8	0.55
6	10	0.712

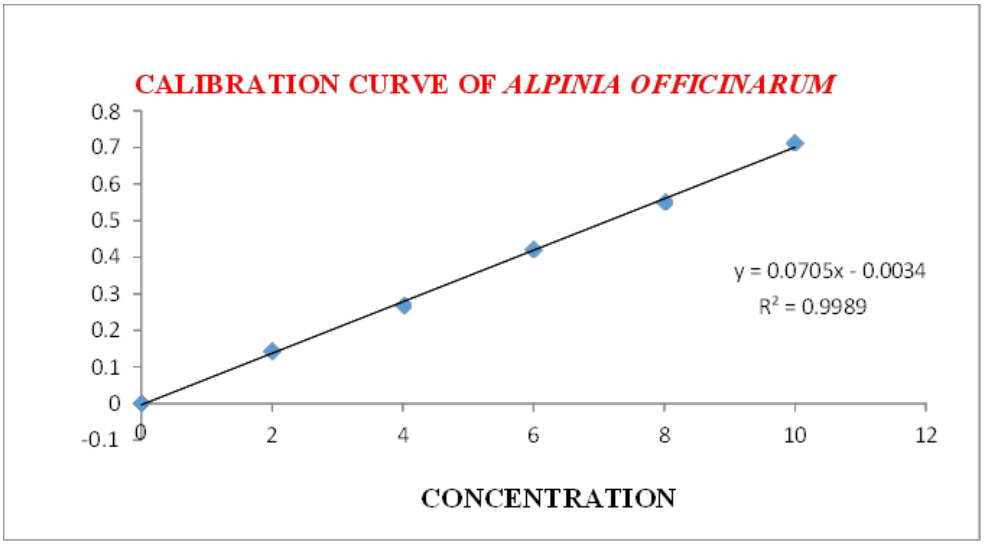


Fig:9 CALIBRATION CURVE OF *ALPINIA OFFICINARUM*

Tab:18 Drug content for *Alpinia officinarum*

S.no	Formulation code	Drug content
1	F1B1	89.4%
2	F1B2	87.2%
3	F1B3	85.4%
4	F1B4	90%
5	F2B1	91.6%
6	F2B2	94%
7	F2B3	87.6%
8	F2B4	86%
9	F3B1	86.2%
10	F3B2	87.4%
11	F3B3	81%
12	F3B4	87.6%

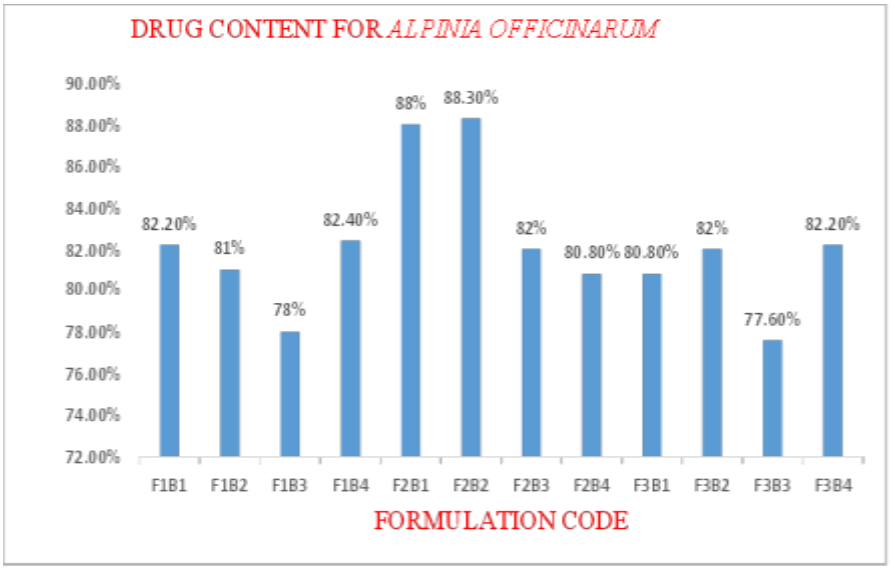


Fig:10 Drug content for *Alpinia officinarum*

Tab:19 CALIBRATIONCURVEOF *SANGU PARPAM*

S.NO	CONCENTRATION (µg/ml)	ABSORBANCE At 270.50 nm
1	0	0
2	2	0.122
3	4	0.271
4	6	0.42
5	8	0.58
6	10	0.69

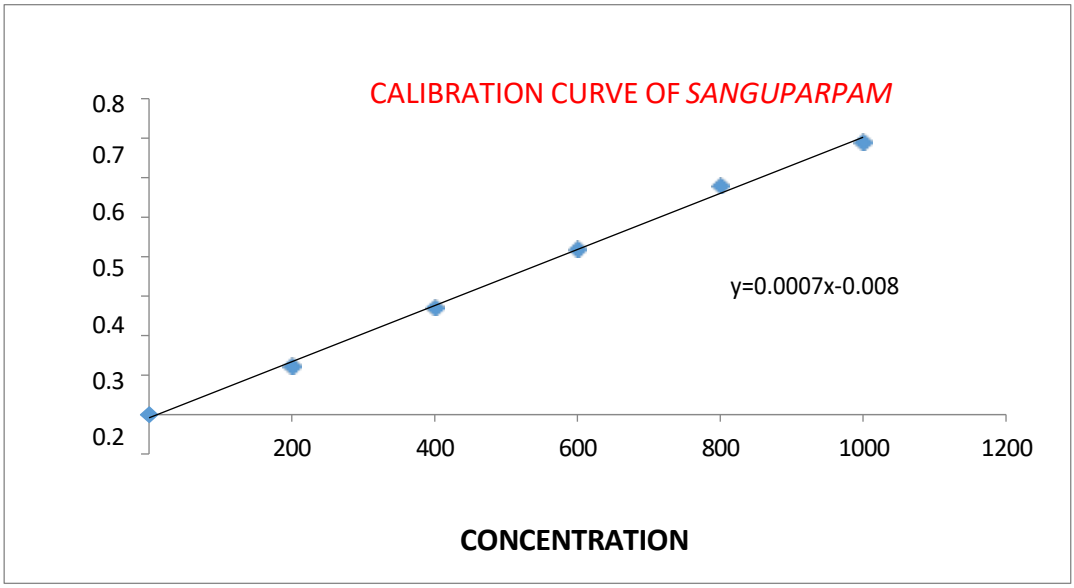


Fig:11 CALIBRATIONCURVEOF *SANGU PARPAM*

Tab:19 Drug content for *Sangu parpam*

S no	Formulation code	Drug content
1	F1B1	82.2%
2	F1B2	81%
3	F1B3	78%
4	F1B4	82.4%
5	F2B1	88%
6	F2B2	88.3%
7	F2B3	82%
8	F2B4	80.8%
9	F3B1	80.8%
10	F3B2	82%
11	F3B3	77.6%
12	F3B4	82.2%

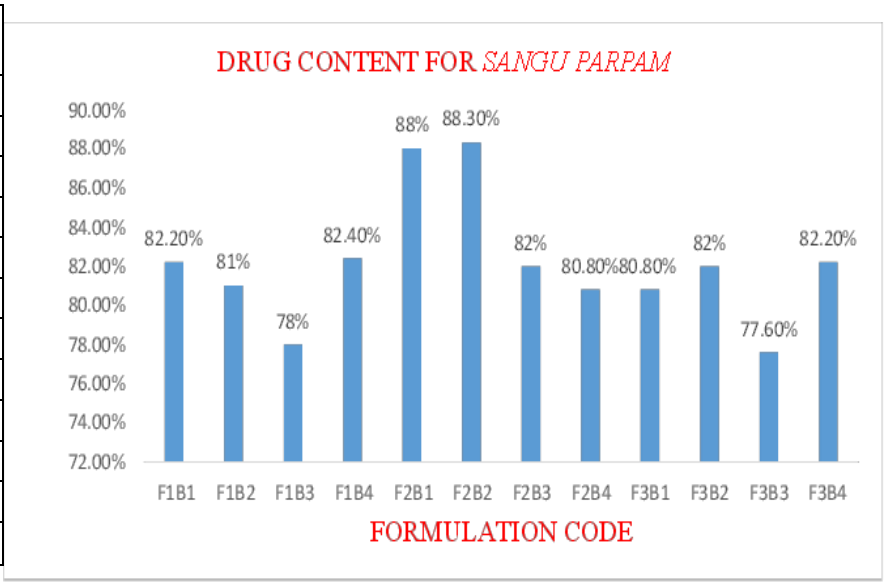


Fig:12 Drug content for *Sangu parpam*

i. pH OF MOUTHWASH FORMULATION

Tab: 20 pH of Mouthwash formulation

S.no	Mouth wash formulation	pH range
1	F1B1	7.31
2	F1B2	7.19
3	F1B3	7.00
4	F1B4	7.04
5	F2B1	6.46
6	F2B2	6.70
7	F2B3	6.86
8	F2B4	6.96
9	F3B1	7.23
10	F3B2	7.19
11	F3B3	7.20
12	F3B4	7.20

Fig:13 A graph illustrating the pH range of various mouthwash formulations loaded with different bases, obtained through the Magnetic stirring, probe sonication, Ultrasonication, and Magnetic stirring methods, is presented.

Tab: 21 Viscosity of Mouthwash formulation

S.no	Mouthwash formulation	Viscosity
1	F1B1	3 .0 cp
2	F1B2	3.6 cp
3	F1B3	3.6 cp
4	F1B4	3.3 cp
5	F2B1	2.7 cp
6	F2B2	3.0 cp
7	F2B3	3.6 cp
8	F2B4	3.9 cp
9	F3B1	3.6 cp
10	F3B2	2.7 cp
11	F3B3	3.6 cp
12	F3B4	3.3 cp

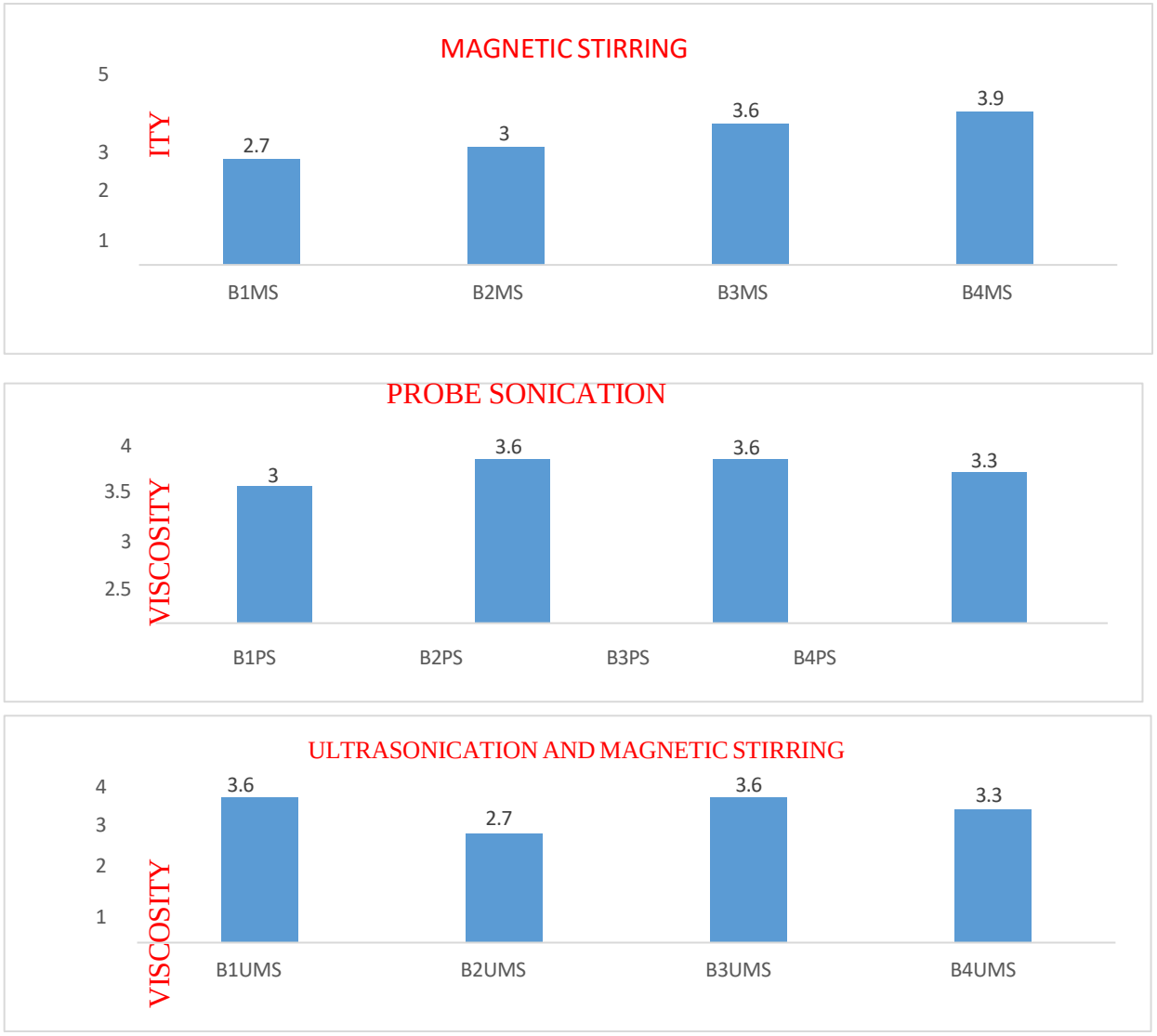


Fig:14 A graph illustrating the viscosity range of various mouthwash formulations loaded with different bases, obtained through the Magnetic stirring, probe sonication, Ultrasonication, and magnetic stirring method, is presented

j. DROPLET SIZE DISTRIBUTION, ZETA POTENTIAL, AND STABILITY OF OPTIMIZED FORMULATION IN EACH METHOD

F2B2

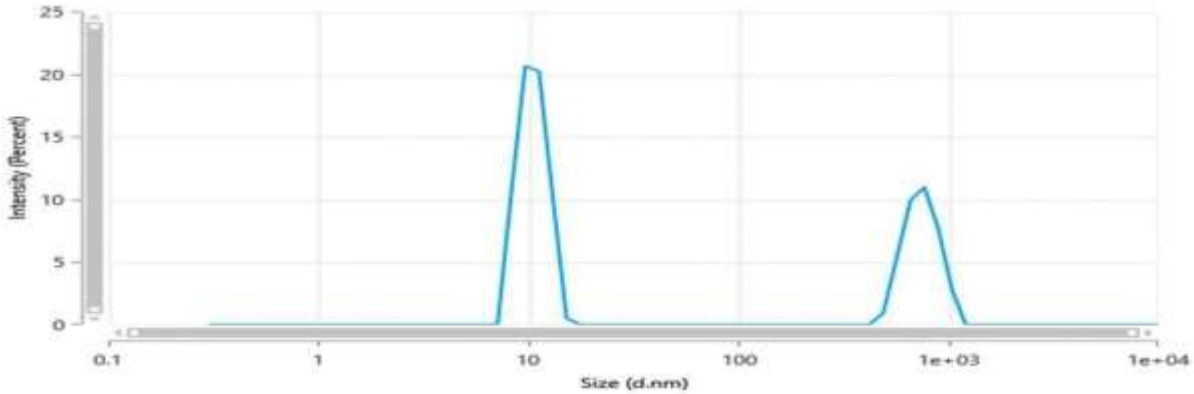


Fig:15 Droplet size distribution and stability of F2B2 Tab:22 Droplet size distribution and stability of F2B2

Name	Mean	Standard deviation	RSD	Minimum	Maximum
z- Average	299.1	-	-	299.1	299.1
Polydispersity index	0.4104	-	-	0.4104	0.4104
Peak1mean by intensity ordered by area (nm)	10.54	-	-	10.54	10.54
Peak1mean by intensity ordered by area (%)	62.3	-	-	62.3	62.3
Peak2meanby intensity ordered by area(nm)	750.6	-	-	750.6	750.6
Peak2meanby intensity ordered by area (%)	37.7	-	-	37.7	37.7

F2B2

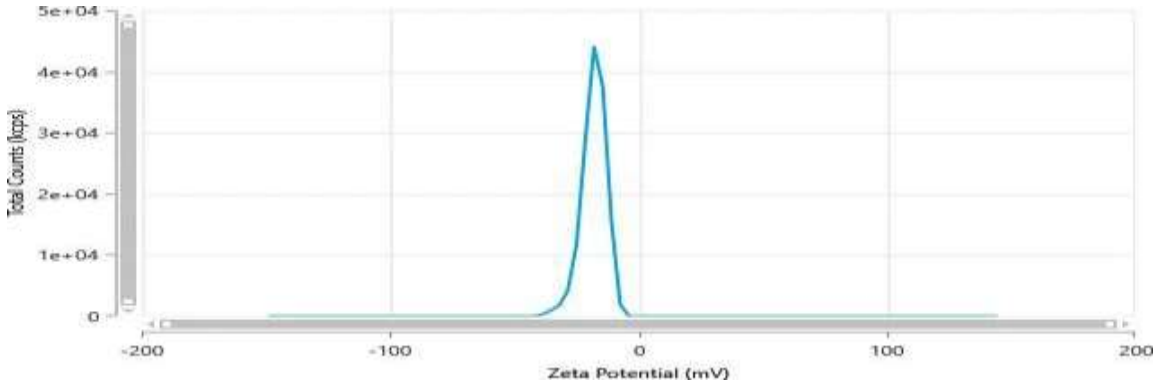


Fig:16 Zeta potential of F2B2

Tab: 23 Zeta potential of F2B2

Name	Mean	SD	RSD	Minimum	Maximum
Zeta Potential(mV)	-18.19	-	-	-18.19	-18.19
Conductivity(mS/cm)	0.4643	--	-	0.4643	0.4643
Wall Zeta Potential(mV)	-25.7	-	-	-25.7	-25.7
Quality Factor	2	-	-	2	2
Zeta Peak 1 Mean(mV)	-18.19	-	-	-18.19	-18.19

F3B4

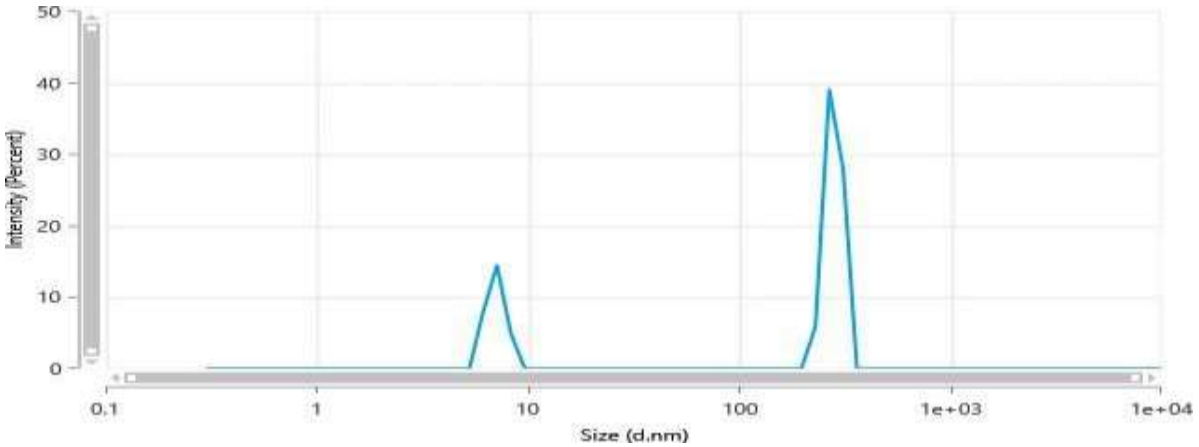


Fig:17 Droplet size distribution and stability of F3B4 **Tab:24 Droplet size distribution and stability of F3B4**

Name	Mean	Standard deviation	RSD	Minimum	Maximum
z- Average	844.1	-	-	844.1	844.1
Polydispersity index	0.6341	-	-	0.6341	0.6341
Peak1meanby intensity ordered by area (nm)	280.9	-	-	280.9	280.9
Peak1meanby intensity ordered by area (%)	72.73	-	-	72.73	72.73
Peak 2 mean by intensity ordered by area(nm)	7.055	-	-	7.055	7.055
Peak 2 mean by intensity ordered by area (%)	27.27	-	-	27.27	27.27

F3B4

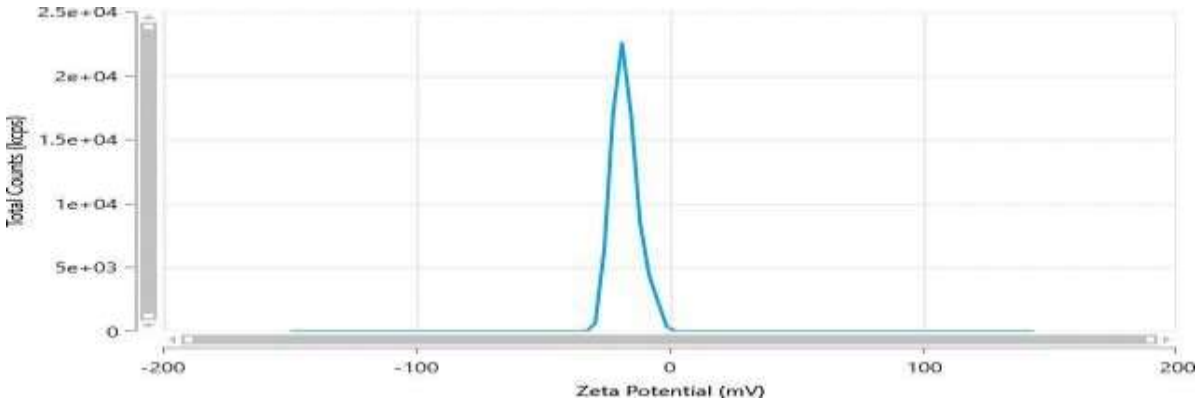


Fig:18 Zeta potential of F3B4

Tab:25 Zeta potential of F3B4

Name	Mean	Standard deviation	RSD	Minimum	Maximum
Zeta Potential (mV)	-17.53	-	-	-17.53	-17.53
Conductivity (mS/cm)	0.2191	-	-	0.2191	0.2191
Wall Zeta Potential(mV)	-21.71	-	-	-21.71	-21.71
Quality Factor	1.092	-	-	1.092	1.092
Zeta Peak 1Mean (mV)	-17.53	-	-	-17.53	-17.53

F1B4

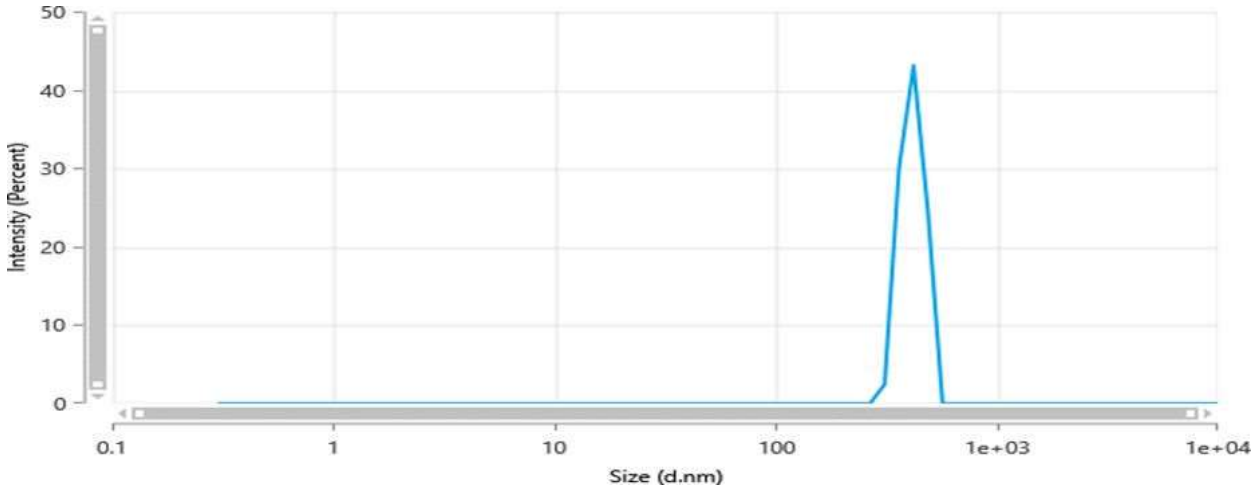


Fig:19 Droplet size distribution and stability of F1B4

Tab:26 Droplet size distribution and stability of F1B4

Name	Mean	Standard deviation	RSD	Minimum	Maximum
z- Average	582.4	-	-	582.4	582.4
Polydispersity index	0.8218	-	-	0.8218	0.8218
Peak1meanby intensity ordered by area(nm)	527.14	-	-	527.14	527.14
Peak1meanby intensity ordered by area (%)	41.36	-	-	41.36	41.36
Peak2mean by intensity ordered by area(nm)	11.12	-	-	11.12	11.12
Peak 2 mean by intensity ordered by area (%)	36.69	-	-	36.69	36.69

F1B4

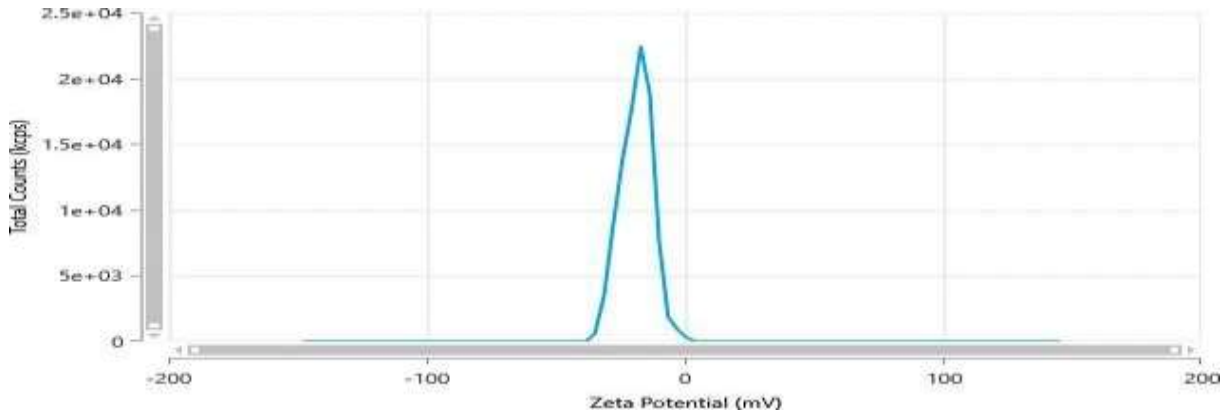


Fig:20 Zeta potential of F1B4 **Tab:27 Zeta potential of F1B4**

Name	Mean	Standard deviation	RSD	Minimum	Maximum
Zeta Potential(mV)	-18.7	-	-	-18.7	-18.7
Conductivity(mS/cm)	0.2938	-	-	0.2938	0.2938
Wall Zeta Potential(mV)	-25.03	-	-	-25.03	-25.03
Quality Factor	1.713	-	-	1.713	1.713
Zeta Peak 1Mean(mV)	-18.17	-	-	-18.17	-18.17

k. SEM analysis:

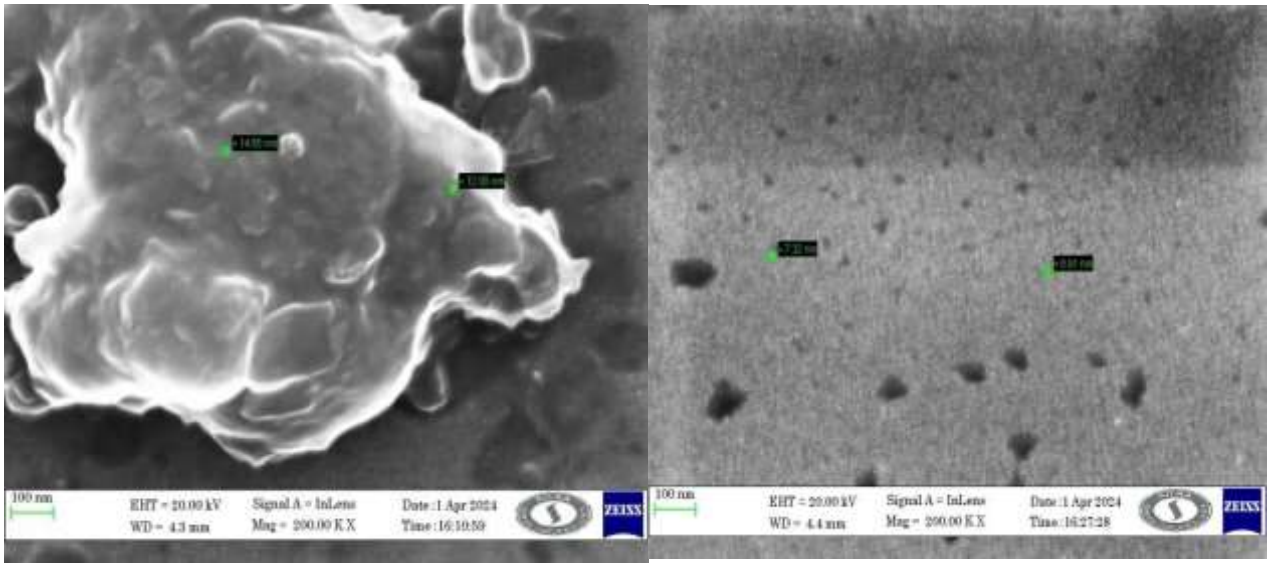
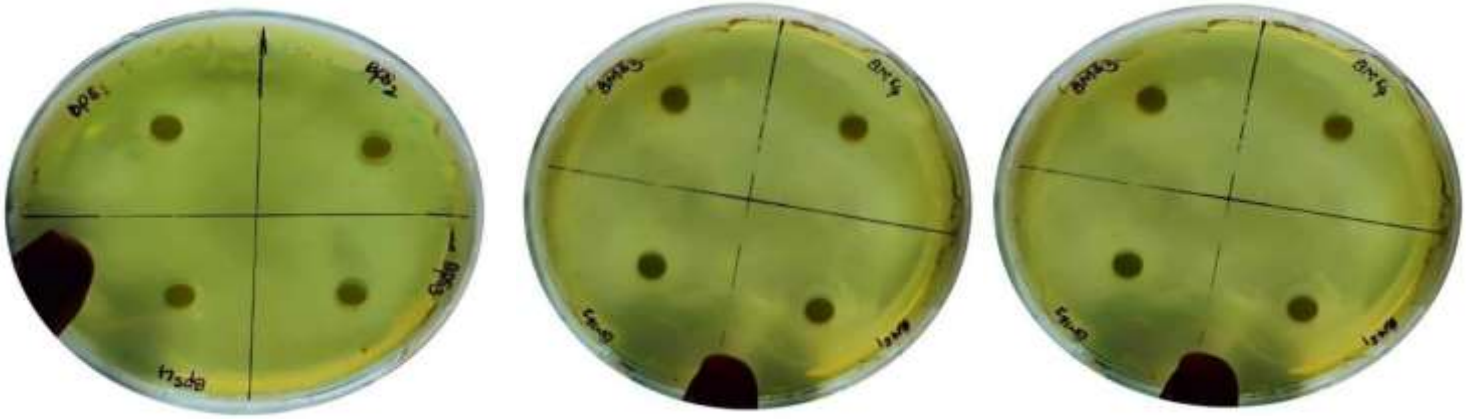


Fig:21 SEM image for F2B2

l. EVALUATION OF ANTI-BACTERIAL ACTIVITY FOR THE OPTIMIZED FORMULATION IN EACH METHOD



F1B4 **F2B2** **F3B4**

Fig:22 ZOI for optimized formulation

5. **STATISTICAL ANALYSIS**

Statistical analysis using GraphPad Prism version 10.0 software showed significant differences in the zone of inhibition (ZOI) values among the tested mouthwash formulations, compared to standard and solvent controls (p-value = 0.0002). Stability studies conducted over one month at refrigerated and room temperatures indicated consistent physical appearance, pH, and viscosity across formulations. No significant changes were observed in color, aroma, pH, or viscosity, affirming the stability and suitability of the formulations for oral use.

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